

**DEPARTMENT OF HEALTH AND HUMAN SERVICES  
NOTE TO FILE (BNF0046)**

**Keywords:**

Canola; glufosinate (herbicide) tolerance; phosphinothricin acetyltransferase (PAT).

**Background**

In a submission dated May 29, 1997, AgrEvo provided summary information to support their safety assessment of genetically modified canola (*Brassica napus*), specifically transformation event T45 and progeny derived therefrom. As a supporting document, this submission also contained the nutritional and safety assessment of canola line HCN92, which was the subject of a previous consultation.

**Intended Effect and Food/Feed Use**

The intended technical effect of this genetic modification of canola is to confer tolerance to the herbicidal compound phosphinothricin, the active moiety of glufosinate ammonium. Canola (oilseed rape) is grown for its seed, which, in turn, is further processed to yield oil and meal for human food and animal feed, respectively. Canola is a trademark term, and strict standards (lower levels or elimination of the anti-nutritional factors erucic acid and glucosinolates) have been accepted for canola oil and canola meal.

According to AgrEvo, their T45 canola has been modified to express a synthetic version of the *pat* gene, similar to the *pat* gene isolated from *Streptomyces viridochromogenes*. The *pat* gene encodes the phosphinothricin acetyltransferase (PAT) protein, which reportedly confers tolerance to the herbicide glufosinate ammonium.

**Molecular Alterations and Characterization**

AgrEvo described the identity and function of the genetic material introduced into canola by the *Agrobacterium tumefaciens*/Ti plasmid-mediated transformation system. The synthetic *pat* gene was transformed into canola using the vector pHoe4/Ac, derived originally from the standard plant transformation vector pPCV002. The constitutive expression of the *pat* gene in canola occurs through the incorporation of a promoter derived from the cauliflower mosaic virus 35-S RNA gene. This *pat* expression cassette was originally constructed in a pUC-based plasmid system and inserted into the modified Ti plasmid between the left and right border sequences to form pHoe4/Ac. The pHoe4/Ac vector contains an *Escherichia coli* origin of replication, but lacks a BOM (basis of mobilization) site, rendering it immobilisable to other bacteria.

AgrEvo presented data and information that allowed the firm to conclude that 1) a single copy of the T-DNA was incorporated into the plant genome; 2) the insert remained stably integrated in the plant genome through successive generations and was inherited in accordance to the Mendelian single gene model; and 3) there has not been transfer of plasmid DNA from outside the left or right border of the T-DNA region of the transformation vector.

### Expressed Protein

One new protein, the enzyme phosphinothricin acetyltransferase (PAT), is expressed in T45 canola. AgrEvo stated that the PAT enzyme confers the glufosinate-tolerant phenotype on T45 canola because it acetylates glufosinate ammonium (ammonium salt of L-phosphinothricin), the active ingredient in broad spectrum, nonselective herbicides such as Liberty<sup>TM</sup>, Harvest<sup>R</sup>, and Ignite<sup>R</sup>, rendering glufosinate ammonium inactive as a herbicide. AgrEvo additionally stated that acetyltransferases, which are found throughout nature, have a common enzymatic function (transfer of an acetyl group from acetyl CoA to a substrate) but differ in substrate specificity.

AgrEvo presented data that allowed the firm to conclude that the PAT protein is not present in processed canola oil (which is the only food product for human consumption) and that denatured (i.e., inactive) PAT protein is present in processed canola meal (which is used for animal feed) at a level of 2-5 ppm (0.005% of total protein in meal). AgrEvo also presented data and information that allowed the firm to conclude that 1) the PAT protein is not known to be an allergen and does not bear sequence homology to known allergens and toxins; 2) PAT enzymatic activity is destroyed during the first stages of processing to produce edible oil and meal; and 3) PAT enzymatic activity is destroyed under simulated gastric conditions.

### Compositional Analysis

AgrEvo presented data that allowed the firm to conclude that the composition of seed from a canola line derived from T45 canola is equivalent to the composition of traditional canola. AgrEvo presented data concerning seed composition, including oil, protein, ash, crude fiber, calcium, phosphorous, and phytosterol content.

In their submission, AgrEvo stated that 1) two components (i.e, erucic acid and glucosinolates) have been associated with nutritional and palatability concerns in rapeseed; 2) "canola" is a trademark term that is presently defined as seed, oil, and meal from *Brassica napus* and *B. rapa* plants that contain no more than 2% erucic acid in the seed oil and no more than 30 umoles/g of aliphatic glucosinolates in the oil-free, moisture-free meal; and 3) a new variety of canola cannot be registered for use in Canada unless it contains less than 1% erucic acid in the seed oil and less than 20 umoles/g glucosinolate(s) in the meal. AgrEvo presented data that allowed the firm to conclude that 1) erucic acid levels in oil extracted from seed from a canola line derived from T45 canola, grown over multiple years and in multiple locations, are lower than 1%; and 2) glucosinolate levels in meal processed from seed from a canola line derived from T45 canola, grown over multiple years and in multiple locations, are less than 20 umoles/g. AgrEvo also presented data that allowed the firm to conclude that the levels of the anti-nutritive substance phytate were similar in meal processed from seed from a canola line derived from T45 canola and commercially available canola lines.

Conclusions

AgrEvo has concluded that canola containing transformation event T45 is not materially different in composition, nutrition, and safety from canola currently grown, processed, marketed, and consumed for animal feed or human food. At this time, based on AgrEvo's description of its data and analyses, the Agency considers AgrEvo's consultation on canola oil and meal from varieties containing transformation event T45 to be complete.

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